

5 The following oligonucleotides were used in the construction of plasmids.

Table 2: Oligonucleotides Utilized For <i>lovE</i> Variant Cloning	
MO664	(5'GGCCATGGAGGCCGCTAGCTCGAGTCGACGGCCTAGGTGGCCAGCT3') (SEQ ID NO:1)
MO665	(5'GGCCACCTAGGCCGCTCGACTCGAGCTAGCGGCCTCCATGGCCGTAC3') (SEQ ID NO:2)
MO666	(5'GGCGGCCGCTCTAGAACTAGTCTCGAGGGTACC3') (SEQ ID NO:3)
MO667	(5'GGTACCCTCGAGACTAGTTCTAGAGCGGCCGCC3') (SEQ ID NO:4)
MO1794	(5' CACAGCGGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:5)
MO1793	(5' CACCACTAGTACGCGGGCTGATTTCGAC3') (SEQ ID NO:6)
MO1785	(5' CACCACTAGTTATACATTATATAAAGTAATGTG3') (SEQ ID NO:7)
MO1786	(5' CACAGGATCCGTCATCTTTGCCTTCGTTTATC3') (SEQ ID NO:8)
MO195	(5' CGCGGATCCTATTGAACAAGATGGATTGCAC3') (SEQ ID NO:9)
MO196	(5' CCGGAATTCAGAAGAACTCGTCAAGAAG3') (SEQ ID NO:10)
MO841	(5' ACAAAAAAGCAGGCTCCACAATGGCTGCAGATCAAGGTAT3') (SEQ ID NO:11)
MO842	(5' ACAAGAAAGCTGGGTTCATGGAGGAATATTGTTGA3') (SEQ ID NO:12)
MO2278	(5' GGGGATCCAATCGAGGTCCACGACCAGT3') (SEQ ID NO:13)
MO343	(5' GGGGACAAGTTTGTACAAAAAAGCAGGCT3') (SEQ ID NO:14)
MO2273	(5' GGGGATCCGCCAATGGTCCCGTTCAAAC3') (SEQ ID NO:15)
MO2274	(5' ACAAGAAAGCTGGGTTCACAGAATGTTTAGCTCAA3') (SEQ ID NO:16)
MO344	(5' GGGGACCACTTTGTACAAAGAAAGCTGGGT3') (SEQ ID NO:17)
MO2624	(5' GCGATGCCCCAAGCGCAAGCTACGCCAATCCAGGG3') (SEQ ID NO:18)
MO2654	(5' CGTCGCGCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:19)
MO2680	(5' GGACCTTTGCAGCATAAATTACTATACTTCT3') (SEQ ID NO:20)
MO2686	(5' GGCGGTCCATTCGCCATTCAGGCTGCGCAACTGT3') (SEQ ID NO:21)
MO2681	(5' TAAAACTCTTGTTTTCTTCTTTCTCTAAAT3') (SEQ ID NO:22)
MO2700	(5' CAGTGAGCGCGCGTAATACGACTCACTATAGGGCGA3') (SEQ ID NO:23)
MO2701	(5' ATACTTCTATAGACACACAAACACAAATACACACAC3') (SEQ ID NO:24)
MO107	(5' CGCGGATCCCGTCGTTTACAAAC3') (SEQ ID NO:25)
MO197	(5' CCAAGCTTATTATTTTGTACACCAGACCAA3') (SEQ ID NO:26)
MO1293	(5' GGAAGATCTAGCATCGTGGCCAATTTCTTCTAGTTT3') (SEQ ID NO:27)
MO1294	(5' ATAAGAATGCGGCCGCTCAACCTTCCCATTGGGGCGTTTGC3') (SEQ ID NO:28)
MO1787	(5' CACAGGATCCAGCATTATTAATTTAGTGTGTGTATTT3') (SEQ ID NO:29)
MO1788	(5' CACCACTAGTCTCGAGCAGATCCGCCAG3') (SEQ ID NO:30)
MO1793	(5' CACCACTAGTACGCGGGCTGATTTCGAC3') (SEQ ID NO:31)
MO1794	(5' CACAGCGGCCGCTCAACCTTCCCATTGGGGC3') (SEQ ID NO:32)
MO511	(5' GGCCATCGATACAAGTTTGTACAAAAAAGCTGAAC3') (SEQ ID NO:33)
MO540	(5' GGCGCCCTATTACACCACTTTGTACAAAGAAAGC3') (SEQ ID NO:34)
MO1985	(5' CACACGTCTCCGGCCTCAACCTTCCCATTGGGGCG3') (SEQ ID NO:35)

NO:35)
MO1986 (5' CACACAGATCTCGTGGCCAATTTCTTCTAGTTTGA3') (SEQ ID NO:36)
MO1992 (5' CACACGGATCCACAATGTTACGTCCTGTAGAAACCCC3') (SEQ ID NO:37)
MO1993 (5' CACAGCGGCCGCTTCATTGTTTGCCTCCCTGCTG3') (SEQ ID NO:38)
MO316 (5' GCGGCCGCGGCGCCCGGCCCATGTCAACAAGAAT3') (SEQ ID NO:39)
MO318 (5' CCGCGGCCGAGTGGAGATGTGGAGT3') (SEQ ID NO:40)

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Plasmid MB2254 contains the *lovFp-HIS3p-neo* reporter gene flanked by *URA3* sequence. First primers MO664 (SEQ ID NO:1) and MO665 (SEQ ID NO:2) were annealed and

10 inserted into the *KpnI-SacI* sites of plasmid pBluescript II KS (Stratagene,). The resulting vector, MB1038, contains a *SalI* site in the polylinker. Next, the *SpeI-XhoI* fragment from pJL164 (Brachmann et al. *Yeast* **14**:115-132 (1998)) containing a deletion of the *URA3* gene with

15 additional flanking sequences was inserted into the *NheI-SalI* sites of MB1038 to create MB1053. Primers MO666 (SEQ ID NO:3) and MO667 (SEQ ID NO:4) that contain multiple restriction sites (*NotI*, *XbaI*, *SpeI*, *XhoI* and *KpnI*) were then annealed together and ligated into the *SmaI* site of

20 MB1053 to create MB1054. Next, the following four fragments were combined in MB1054 to obtain plasmid MB2254. The *lovF* promoter from *A. terreus* genomic DNA was PCR amplified with MO1794 (SEQ ID NO:5) and MO1793 (SEQ ID NO:6) and inserted into MB1054 on a *NotI-SpeI* fragment.

25 The *HIS3* basal promoter from pRS403 (Sikorski and Hieter, *Genetics* **122**:19-27 (1989)) was PCR amplified with primers MO1785 (SEQ ID NO:7) and MO1786 (SEQ ID NO:8) and inserted into MB1054 on a *SpeI-BamHI* fragment. Finally, the *neo* gene (PCR amplified with MO195 (*BamHI*) (SEQ ID NO:9) and

30 MO196 (*EcoRI*) (SEQ ID NO:10) from plasmid pYX11 (Xiao and Weaver, *Nucl. Acids Res.* **25**:2985-2991 (1997)) and *CYC1* terminator sequences (*XhoI-KpnI* fragment from pRS426-GAL-S (Mumberg, et al., *Nucl. Acids. Res.* **22**:5767-5768 (1994)) were first combined in pRS416 (Sikorski and Hieter,

5 *Genetics* 122:19-27 (1989)) and then cut out with *Bam*HI-
KpnI and inserted into MB1054 to create MB2254.

 The *lovFp-HIS3p-neo* reporter in MY2124 can confer
resistance to the drug geneticin (G418). It was
empirically determined that MY2124 (untransformed or
10 transformed with parental plasmids MB2478 (*CYC1-lovE/CEN*)
or MB2848 (*CYC1-lovE/At274/CEN*) was unable to grow on YPD
media supplemented with 100 µg /ml G418. Plasmid MB2478
contains the *CYC1* promoter operationally linked to the
entire *A. terreus lovE* open reading frame. The *CYC1*
15 promoter is a relatively weak promoter and thus the *lovE*
ORF in MB2478 was expressed at low levels. MB2478 was the
parental vector plasmid for creating full length *lovE*
variants. Plasmid MB2848 contains the *CYC1* promoter
operationally linked to a chimeric open reading frame
20 consisting of the *A. terreus lovE* DNA binding domain fused
to the carboxy-terminal portion of the *At274* gene (U.S.
Serial No. 60/257,431, filed December 22, 2000).

 MB2848 was used to create *lovE* variants in which the
DNA binding domain was not mutated. Both MB2478 and
25 MB2848 contain yeast *CEN* and autonomously replicating
sequences and both are maintained at 1-2 copies per cell.
In contrast to strains transformed with MB2478 or MB2848,
strains transformed with plasmid MB1644 (*TEF1-lovE/2*
micron) were able to grow on G418-supplemented YPD media.
30 The *lovE* gene of MB1644 is under control of the
constitutively strong *S. cerevisiae TEF1* promoter. MB1644
contains a 2-micron origin for high-copy replication in
yeast. An objective of these studies was to identify *lovE*
variants which when expressed at low levels could confer
35 G418 resistance similar to the highly expressed wild-type
lovE molecule of MB1644. *S. cerevisiae* expression vectors
used in these studies were constructed as follows.

 MB968 is a low copy *S. cerevisiae URA3* based
expression vector. MB968 was created by inserting the
40 *EcoRV* fragment (containing the destination cassette) from
gateway pEYC7201 (Invitrogen™, Carlsbad, CA) into